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β-GLUCURONIDASE AND RELATED GLYCOSIDASES DEMONSTRATED BY INDOLYL SUBSTRATES IN LYMPHATIC TISSUE

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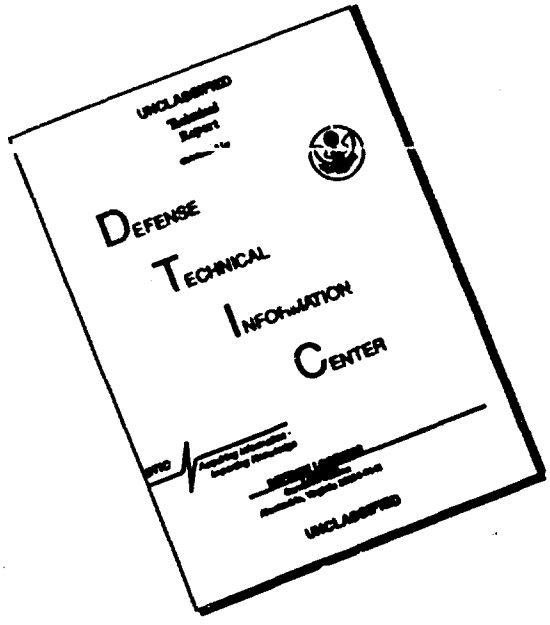

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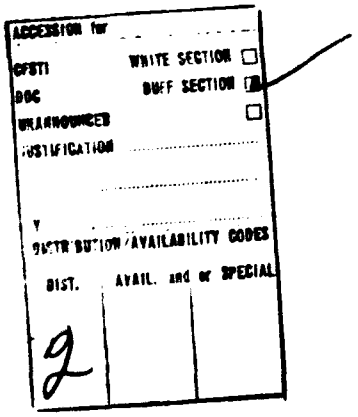

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Project 1T013001A91A

April 1968

ABSTRACT

Histochemical and cytochemical techniques for detecting enzyme activity have been difficult to apply to the lymphatic system. We have been able to demonstrate tissue glycosidases with techniques that show sharp intracellular localization and that are highly specific, although the heterogeneity and functional changes in component cells may still present interpretive difficulties. We have developed halogen-substituted indolyl substrates for β-galactosidase, β-glucosidase, β-2-deoxyglucosidase, and β-fucosidase. The most recently developed substrate for β-glucuronidase is 5-bromo-4-chloroindol-3-yl-β-D-glucopyruroniside. The final reaction product in all cases is identical: 5,5'-bromo-4,4'-chloroindigo. This intensely blue-green, finely granular precipitate is substantive and insoluble so that tissues can be dehydrated and permanently mounted. Specific inhibition for each substrate has been demonstrated with analogue lactones.

Differences in the distribution and intensity of the resultant staining in the various cells and tissues of the lymphoid system for these enzymes offer a unique opportunity to study the development of functional changes in reticular cells, including enzymatic induction and antigenic response.

I. INTRODUCTION

Recently we have developed a new indolyl substrate for β-glucuronidase, viz: 5-bromo-4-chloroindol-3-yl-β-D-glucopyruroniside.[1] The final reaction product after hydrolysis by β-glucuronidase in tissue is an insoluble, fine bluish-green precipitate, 5,5'-bromo-4,4'-chloroindigo. This substrate is identical to the other histochemical substrates that contain the same halogen-substituted indolyl moiety. The four indolyl substrates for tissue glycosidases developed by us are those for the demonstration of β-galactosidase, β-glucosidase, 2-deoxyglucosidase, and β-fucosidase; the detailed methods for the reactions have been reported previously.[3-5] We have also demonstrated reaction specificity related to the rotation and configuration of the glycon.[6] The differences in configuration and substitution for these substrates can be seen in Figure 1. The 2-deoxyglucose (not shown) differs from glucose only in C-2 position.

The purpose of the present study is to compare the results of β-glucuronidase reactions with those of other glycosidases in fixed and free components of the reticuloendothelial system. Rat and rabbit tissues were used. Our results indicated that our indolyl histochemical and cytochemical methods can readily be applied to lymphoid tissue as useful techniques in the study of immunopathology.

II. RESULTS

A. GENERAL DISTRIBUTION

The reticular cells of the lamina propria of the intestine showed activity for all the glycosidases except 2-deoxyglucosidase. The substrate for fucosidase showed the most intense reaction, but strong to moderate activity was also found for galactosidase, glucuronidase, and glucosidase, in the order listed. The spleen showed the best reaction with the galactoside and good reactions with the glucuronide. The degree of reactivity for galactosidase and glucuronidase was also found in the mesenteric lymph node, Peyer's patches, and peripheral lymph nodes, such as the popliteal and axillary nodes. A search for enzymes in the thymus showed only a few cells, usually one or two, and in most cases no reactive cells. The only enzymes demonstrated were glucuronidase and galactosidase.

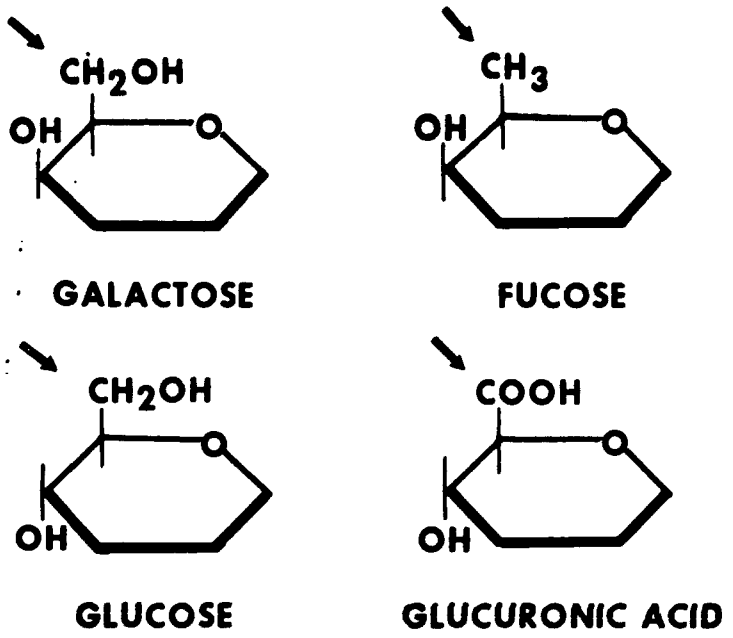


Figure 1. Differences in the C-4 Hydroxyl and C-6 Terminal Group (arrow) in Related Sugars. The indolyl moiety in each substrate is attached to the carbohydrate in the D-configuration by a β-linkage at C-1.

The lymphatic tissue most uniformly involved was the lamina propria of the small intestine, spleen, and popliteal components. The reaction was for fucosidase, which was extremely strong in the lamina propria of the intestine, but entirely negative in all other lymphoid tissue. This is of interest when one compares the glycon moiety of fucoside with that of galactoside (Fig. 1). Although glucosidase was found in all lymphatic tissue examined, reactive cells were not abundant. The 2-deoxyglucosidase was found in the splenic cortex, where it was associated with cells showing phagocytosis or injury. The distribution of tissues with glucuronidase activity compared with the other glycosidases is listed in Table 1.

TABLE 1. POSITIVE OVERALL ENZYME REACTIONS IN VARIOUS LYMPHOID STRUCTURES

Structure	β-Glucuronidase	β-Galactosidase	β-Glucosidase	β-Fucosidase
Lamina propria	+++	++++	++	++++++
Spleen	+++	++++	+	0
Popliteal	++	+++	+	0
Mesenteric	+	++	-a/	0
Peyer's patch	0	+	-	0
Thymus	+	+	-	0

a. - = not examined.

B. SPECIAL DISTRIBUTIVE FEATURES

Reactive cells were not found in lymphoid follicles in these presumably normal tissues. In the spleen, reactions were seen only in the reticular cells of the red pulp, some of the cortical sinuses, and cells adjacent to the trabeculae. In the popliteal lymph node, where reactions were especially strong, positive cells were particularly numerous in the medullary cords and sinuses. In the medullary cords, reactive cells were associated with the sinus reticulum, and staining was present in the sinuses, both in cells attached to the walls and apparently free cells in the sinuses. Most of these free cells in the sinuses were large monocytes. This pattern was typical of the findings in other lymph nodes, but to a much lesser extent. The thymus was a special case because the medulla was stained prominently. Under pathological conditions, both glucuronidase and galactosidase increased mark dly.

C. INTRACELLULAR FEATURES

Indolyl substrates are ideally suitable for cytologic studies. This is because of the fine, particulate nature of the final reaction product, its substantivity, and the reaction specificity determined by analogue lactone inhibitors. Glucuronidase and galactosidase can readily be demonstrated in free monocytic derivatives of the lymphatic system. Figure 2 shows glucuronidase and Figure 3 shows galactosidase in peritoneal mononuclear cells. The monocytic cells were placed in tissue culture for 4, 24, or 72 hours and incubated in their respective substrates. Specificity was determined by inhibition with lactones derived from the same glycoside. Increased enzyme activity was directly related to the development of intracellular organelles. The two enzymes had similar distribution in the cytoplasm, but the nuclei gave negative results. The distribution of the finely granular indigo precipitate, indicating enzyme activity, can be readily appreciated in Figure 3, C. No diffusion was seen. The intracellular localization of these reactions is identical in alveolar macrophages, and the activity is several times greater.

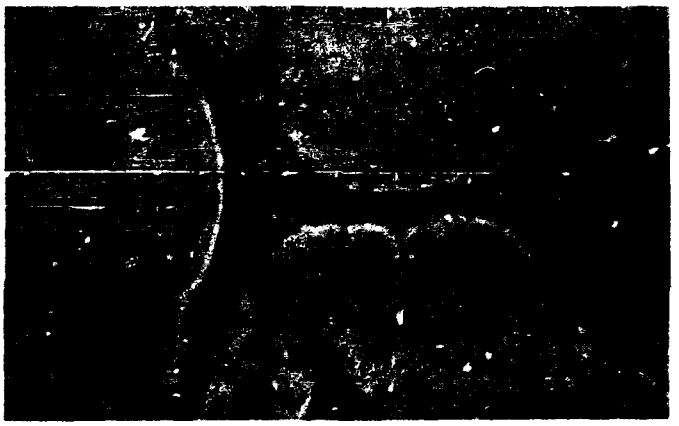
III. CONCLUSION

Histochemical reactions for β-galactosidase, β-glucosidase, β-2-deoxyglucosidase, β-fucosidase, and now β-glucuronidase have been demonstrated with the use of halogen-substituted indolyl substrates in the various fixed and free derivatives of reticular cells in the lymphatic system. The reaction is highly specific, and the reaction product is finely particulate and substantive. The usefulness of these techniques in work with the reticuloendothelial system is shown by the wide distribution and selective characteristics of the reactivity of these cells in the normal host.

Our recent studies indicate that β-glucuronidase reactions in lymphatic tissue are, in general, comparable to those for the other indolyl glycosidases, and that these histochemical methods will be useful in the study of the normal and immunologically altered host.

A

B



C

Figure 2. Peritoneal Monocytes Placed in Tissue Culture at 4, 24, and 72 Hours, Respectively. Fixed in glutaraldehyde and incubated to demonstrate β-glucuronidase. The 4-hour sample, A, shows cell, upper left, where cytoplasmic tissue in "hof" shows enzyme nucleus is negative. B (24 hours) and C (72 hours) show progressive increases in enzyme. 2500X.

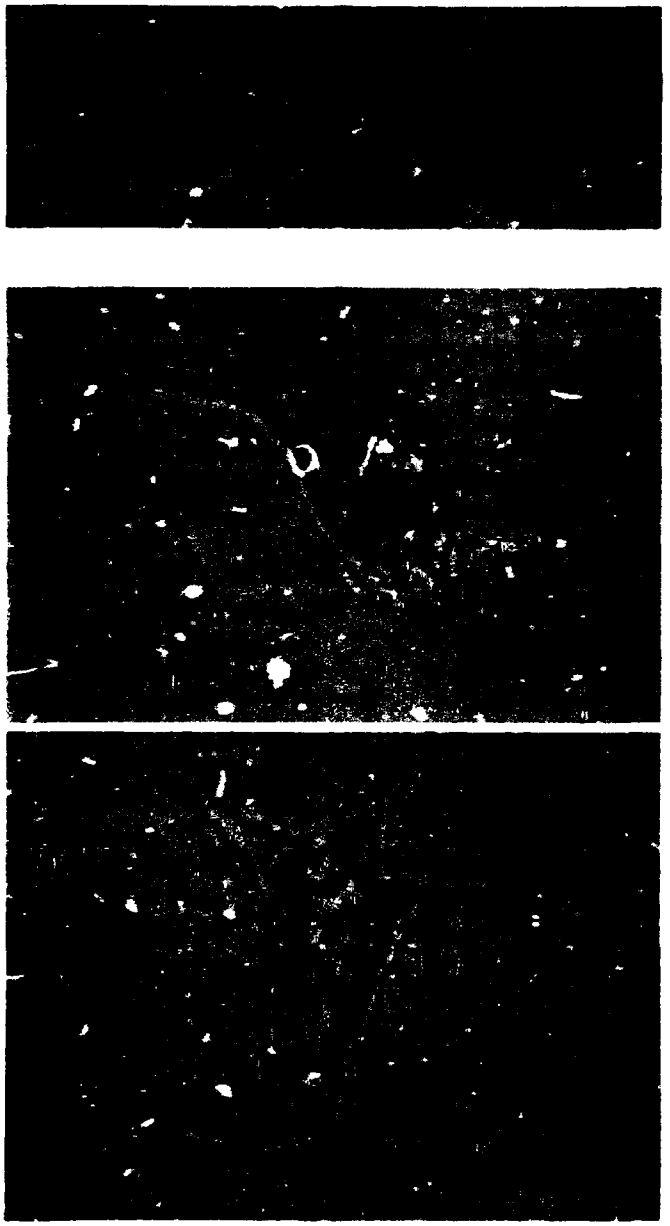


Figure 3. Peritoneal Monocytes Placed in Tissue Culture at 4, 24, and 72 Hours, Respectively. Fixed in glutaraldehyde and incubated to demonstrate β-galactosidase. Progressive increase is seen in enzyme. C shows marked enzyme in cytoplasm. 2500X.

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14. Key Words

Glucuronidase
*Glycosidases
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*Enzyme action
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